Contributions to the understanding of diversity and evolution in the genus *Coreomyces*

HENRIK SUNDBERG
Abstract

The Laboulbeniales is an order of enigmatic ascomycete fungi that are obligate microscopic parasites of arthropods, which are usually non-detrimental. They typically display a high degree of host specificity, and the genus *Coreomyces*, which is the recurring theme of this thesis, is no exception. *Coreomyces* is a small aquatic genus parasitising water boatmen (Corixidae). Members in this genus present something that is unique for Laboulbeniales, position specificity. This means that a parasite is restricted to a specific position on the host. This thesis aims at elucidating the patterns of host and position specificity present in the genus *Coreomyces* by the use of molecular markers. A second aim is to describe new taxa encountered during the course of the project. Finally we wanted to reveal what mating system is present in a member of the Laboulbeniales, something that has never been done before. Understanding the mode of sexual reproduction and to get insights into mating type organisation can give important clues to how the species patterns we observe may have evolved. To achieve these goals we first had to develop a robust and simple molecular methodology that made use of as little material as possible, preferably a single thallus. Our results showed that position specificity indeed exists in this genus, but that it is not as strict as previously presumed, all species we analysed occupied more than one position. Further we were able to show that thalli found in the same position on different hosts constitute the same species, and also that two sister species utilise the same position. We conclude that, in most cases, growth position is more important than host species or host sex in species delimitation. We confirmed the presence of four discrete taxa, two of which were described as new species, *Coreomyces confusus* H. Sundb. et al. and *C. dextrorsus* H. Sundb. et al. Finally we were able to show that *C. macropus* and *C. confusus* are likely to display a homothallic breeding system.

Keywords: ascomycetes, Coreomyces, Corixidae. DNA, DNA extraction, Fungi, genome, host specificity, Laboulbeniales, mating types, MAT-loci, methodology, NGS, parasite, position specificity, systematics, taxonomy

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Till mor, far och Telpa.
‘For in much wisdom is much grief:

and he that increaseth knowledge increaseth sorrow.’

Ecclesiastes 1:18
This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


III Sundberg, H., Kruys, Å., Bergsten, J. Ekman, S. Two new species in the genus *Coreomyces* Thaxt. from Europe. *Manuscript*

IV Sundberg, H., Onut-Brännström, I., Kruys, Å., Ekman, S. Insights into the mode of reproduction in the genus *Coreomyces* Thaxt. (Ascomycota, Laboulbeniales). *Manuscript*

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## Abbreviations

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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>HMG-box</td>
<td>High mobility group box domain</td>
</tr>
<tr>
<td>ITS</td>
<td>Internal Transcribed Spacer</td>
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<tr>
<td>MDA</td>
<td>Multiple Displacement Amplification</td>
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<tr>
<td>mrSSU</td>
<td>Mitochondrial Small Subunit</td>
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<tr>
<td>NGS</td>
<td>Next Generation Sequencing</td>
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<td>nrLSU</td>
<td>Nuclear Large Subunit</td>
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<tr>
<td>nrSSU</td>
<td>Nuclear Small Subunit</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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Introduction

The order Laboulbeniales

Laboulbeniales were first observed by the French entomologist Joseph Alexandre Laboulbène in the 1840's. Although a large order among the ascomycetes, the Laboulbeniales are known to many mycologists only by their name, or perhaps for being insect parasites. Very few can claim to actually have seen these fungi in real life, the reasons being, of course, their size - their microscopic thalli has a general size range of roughly 0.05–1 mm, and also their substrate - arthropods are seldom studied by mycologists. Therefore studies of Laboulbeniales has mainly been conducted by entomologists. But even among these scholars, many have not noticed or even heard of these fungi. So, despite their great diversity and captivating lifestyle they are the least studied of all arthropod-associated fungi (Weir & Beakes 1995). Another reason for the apparent lack of research could be the fact that they generally do not substantially affect their host negatively. Being non-detrimental and therefore regarded of little use in e.g. biological control, they do not receive the same attention as more serious pests do.

Systematic position

Despite being first observed by Laboulbène in the 1840's, the first two species were described by Montagne and Robin in 1853, who named the genus Laboulbenia in honour of the discoverer (Robin 1853). The order Laboulbeniales was established by Lindau (1897). Although they were regarded as fungi already by Robin (1853), there were deviating opinions on what kind of organisms they really were. Interpretations spanned from them being outgrowths of the insect itself, that they were parasitic worms, or red algae, until they eventually were described as being fungi with varying affiliations to Ascomycota, Basidiomycota, and even Zygomycota. It was not until Thaxter described their ascogenous spore development that they finally settled among the Ascomycetes (Thaxter 1896). Although most mycologists in the last century agreed that Laboulbeniales belong to ascomycetes, there have been some more recent objections, for instance Cavalier-Smith (2001) wanted to place them (based on superficial similarities of the holdfast organs) together with the former class Trichomycetes within the Zygomycota.
The position within the Ascomycota was long a source of controversy. Although Engler had established Laboulbeniomycetes already in 1898 (Engler et al. 1897), his definition and systematic placement of the class differed from the modern one based on DNA analyses (Weir & Blackwell 2001). From the 1960s to the 1980s, several researchers revived an old theory, originally proposed by Sachs (1874), that floridean red algae are the ancestors of true fungi (Denison and Carroll 1966, Kohlmeyer 1973, 1975, Demoulin 1975, 1985). Thus Kohlmeyer (1973) suggested a common ancestry of Laboulbeniales and Spathulosporales (a parasite of red algae), since both show the same plesiomorphic traits and superficially resemble each other. This common ancestor was suggested to be a parasitic red alga. Molecular techniques have since demonstrated that fungi do not share a most recent common ancestor with the red algae and that Laboulbeniomycetes should be regarded as a separate class within the Pezizomycotina (Weir & Blackwell 2001). It has also, despite the similarity in appearance, been shown that Laboulbeniales are not closely related to the Spathulosporales (Inderbitzin et al. 2004; Weir & Blackwell 2001). Blackwell & Malloch (1989) suggested a relationship between the Laboulbeniales and Pyxidiophorales due to similarities in morphology and development. The latter are arthropod-dispersed saprobes or mycoparasites on a broad spectrum of substrates. The ancestor of Laboulbeniales may have had a lifestyle similar to that of the Pyxidiophorales, switching between hosts and having both anamorphic and teleomorphic stages. In the course of evolution a shift to relying on a single arthropod host took place. Indeed, Pyxidiophorales was eventually demonstrated to be the closest living relatives of Laboulbeniales (Blackwell 1994, Weir & Blackwell 2001).

Systematically the Laboulbeniales form the class Laboulbeniomycetes together with the small orders Herpomycetales and Pyxidiophorales (Haelewaters et al. 2018). The class is the sister group to the Sordariomycetes, with which they share morphological traits such as perithecia and unitunicate asci with poricidal dehiscence (Schoch et al. 2009).

On a finer taxonomic scale, there have been two major competing classifications. Thaxter (1908) based his classification on antheridial characters and divided the order into two suborders, the Laboulbeniineae and Ceratomyctineae. The former included the two families Peyritschiellaceae and Laboulbeniaceae, whereas in the latter he omitted the family level; the Ceratomyctaceae was later diagnosed by Colla (1934). This antheridium-based classification was prevailing until Tavares (1985) discovered that the families established on antheridial characters were not monophyletic. She proposed instead a classification based on the whole thallus but with main focus on characters of the perithecial wall and perithecial development. In this classification she divided Laboulbeniales into the suborders Herpomycetinae and Laboulbeniinae, the former including the single family Herpomycetaceae and the latter the three families Ceratomyctaceae, Euceratomycetaceae and Laboul-
beniaceae. Recently, two phylogenies of the Laboulbeniales have been published by Goldmann & Weir (2018) and Haelewaters (2018). Goldman & Weir (2018) concluded that what they call “Laboulbeniales proper” comprises five distinct clades and also excludes Herpomycetaceae from the order. Haelewaters (2018) found strong support for three orders within the Laboulbeniomy- cetes: Pyxidiophorales, Laboulbeniales and the new order Herpomycetales. In this phylogeny it was also evident that several higher taxa in the classification of Tavares (1985) are polyphyletic (Haelewaters 2018).

Diversity

The greatest contribution to the knowledge of these fungi was made by Roland Thaxter at the end of the 19th and in the beginning of the 20th century. His research resulted in five large volumes published between 1896 and 1931, and his work is still regarded being the cornerstone in Laboulbeniales research. In total, Thaxter described around 1260 species. Since then the number of described species has risen to around 2200 distributed over 141 genera (Haelewaters 2018). The estimated total species number ranges between 10,000–50,000 species on Coleoptera, and an additional 5,000–25,000 species associated with other arthropods (Weir & Hammond 1997). The ratio of described species (1.5–6% of total estimated) is consistent with the estimations for the Mycota as a whole, where supposedly 4–8% (or 60,000–120,000 out of 1.5 million) are scientifically described (Hawksworth 2001).

All members of the Laboulbeniales are regarded as being obligatory ecto-parasites on their host species, which are mostly insects but hosts are also found among members of the arthropod classes Arachnida and Diplopoda. Among the insects, nine out of thirty insect orders host Laboulbeniales (Isop- tera being counted in Blattodea). Around 80% of the species are found on Coleoptera, 10% on Diptera and the remaining 10% parasite the other seven insect orders plus mites and millipedes (Weir & Blackwell 2005, Santamaria et al. 2017). Comparatively few families within each order host Laboul- beniales, however, and this pattern is repeated as we descend in rank. Even within a group of closely related species some taxa may be hosts, others not. Consequently, it is not surprising that many species of Laboulbeniales show high degree of host specificity. That some species of Laboulbeniales are only able to grow on a limited set of closely related hosts has also been confirmed experimentally. The results from these studies indicate that a biochemical as well as an environmental component is present in the explanation of host specificity, at least in some cases and to a certain extent (e.g. De Kesel 1996, Richards & Smith 1954).

There are also examples of position specificity (i.e. confinement to a certain spot on the host) and sex-of-host specificity (species being restricted to different host sexes) reported (Weir & Blackwell 2005). Position specificity has
been shown to be true in a number of recent studies (e.g. Goldmann & Weir 2012, Sundberg et al. 2018b), but host sex specificity on the other hand has been shown to involve different morphs of the same species growing on male and female hosts (Goldmann & Weir 2012). The different morphs have been explained by different parts of the insect providing nutrients of various kinds and amounts to the fungus. Alternatively the thalli may develop differently in response to the specific external stress that comes with the associated exposure of a particular position and finally that thalli develop different dispersal structures that are adapted for a certain position (Rossi & Kotrba 2004).

The ascospores are transmitted between hosts mainly through direct contact (Richards & Smith 1954, 1955, Whisler 1968, De Kesel 1993, 1995, 1996) or, to a more limited extent, through spore-contaminated host habitat (Arwidsson 1946, Lindroth 1948, Scheloske 1969, De Kesel 1995). The Laboulbeniales are unique compared to other fungi in that their ascospores do not produce germ tubes that develop into hyphae. Instead, the thallus develops directly from the two-celled ascospore by repeated cell divisions. Most genera are monoecious, bearing both male and female sexual organs on the same thallus, but there are also dioecious genera, as well as genera containing monoecious, dioecious and even trioecious species. In the dioecious species male thalli are often reduced, sometimes to as few as three cells (Benjamin 1971, 1986, Santamaria 1998, Weir and Blackwell 2005). The order is also unusual in that they exhibit determinate growth (i.e. they form thalli with a more or less defined number of cells) and do not develop mycelia. They are known to reproduce sexually (Weir & Blackwell 2005) in contrast to most other ascomycete groups where asexual reproduction dominates (Martin et al. 2013). One should however bear in mind that just because asexual life stages have not been observed it does not necessarily rule them out; they may yet to be discovered.

Most members of the Laboulbeniales seem to inflict little or no harm to their host. Indeed, some species do not seem to penetrate the host cuticle at all (Tragust et. al. 2016). Other species, however, have been shown to be detrimental to their host, developing extensive haustoria that penetrate deep into the body of the animal (see e.g. Kamburov 1967, Meola and Tavares 1982). There are also theories that that certain Laboulbeniales are even beneficial to their host species. Perotti and Braig (2011) suggested that some species in the genus Rickia, living on mites, might promote host mating by acting as pheromone receptor enhancing structures in host males, although this study fails to provide experimental evidence for this proposal.
The genus *Coreomyces*

Diversity and systematic position

*Coreomyces* is the single genus of the tribe Coreomyceteae in the family Laboulbeniaceae according to the classification by Tavares (1985). However, the phylogeny of Haelewaters (2018) strongly supports the placement of *Coreomyces* together with the genera *Ceratomyces*, *Chitonomyces* and *Zodiomyces* in an “aquatic clade” that live on aquatic hosts. *Ceratomyces* is according to the classification by Tavares (1985) placed in the family Ceratomycetaceae, whereas the other three genera were placed in the Laboulbeniaceae.

In all, 21 species within *Coreomyces* have been described; from all continents except Australia and Antarctica (MycoBank 2018). Their hosts are various species within the family Corixidae (water boatmen). The species encountered in Europe are: *C. arcuatus* Thaxt., *C. confusus* H. Sundb. et al., *C. corixae* Thaxt., *C. dextrorsus* H. Sundb. et al., *C. elongatus* Speg. and *C. macropus* Thaxt. (Santamaria et al. 1991, Majewski 1994, Santamaria 2003, Sundberg et al. paper III) A fifth species, *C. italicus* Speg., was synonymised to *C. corixae* by Majewski (1994).

Morphology and terminology

The species in the genus *Coreomyces* (Fig. 1) are monoecious and typically have a receptacle composed of a series of superimposed large cells (denoted I–IV) that are terminated by a single perithecium. Below cell IV, there are a varying number of small flattened cells (appendiculate cells) unilaterally bearing filiform, sometimes slightly branched, appendages that may or may not bear simple intercalary antheridia. These appendages often disappear (at least in the European species) as the individual mature. In some specimens there are deviations from the normal number and placement of the large and appendiculate cells. With the exception of the quite common cell IVa, these cells have no designations. The genus *Coreomyces* is unique in the Laboulbeniales in that the perithecium develops inside the distal cells of the receptacle, subsequently destroying them, ending up with the outer cell walls of these cells lining the wall cells of the perithecium (Thaxter 1931, Majewski 1994, Santamaria 2003).

Species descriptions are based on the number and arrangement of the cells in the receptacle and on the characters of the different parts of the thallus such as shape, size and colour of the receptacle and perithecium, position on the host etc. Unlike in most other ascomycetes, the ascospores of the Laboulbeniales have not been considered informative for species recognition since they are more or less alike across even distantly related taxa.
Host and position specificity

The members of the genus *Coreomyces* seem to be not very restrictive in their host choice and are consequently found on both closely and distantly related corixids (Santamaria et al. 1991, Majewski 1994, Sundberg et al. paper III). On the other hand, all the European species exhibit position specificity, and this is probably the case in all members of the genus (Thaxter 1908, 1931, Majewski 1994, Sundberg et al. 2018a, b). Sundberg et al. (2018b) found no sex of host specificity, in accordance with Majewski (1994).

Sex in ascomycetes

Breeding systems and sexual reproduction modes

There are two breeding systems in fungi, bipolar and tetrapolar. The first type is found throughout the fungal kingdom whereas the second is, as far as we know, unique to the Basidiomycota. There are also two principal modes of sexual reproduction; heterothallism and homothallism. The breeding systems and sexual reproduction modes are outlined in Fig. 2. The modes of sexual
reproduction in Laboulbeniales are not known. However, we do know that some species are monoecious and others dioecious. In the latter case it is obvious that these species are also heterothallic, in the former homothallism would be the first that come to mind, but is it necessarily so? To be able to show that a monoecious Laboulbeniales species is also homothallic could be an important step towards understanding how closely related species can coexist in the limited space that a single host provide.

**Mating types of filamentous ascomycetes**

The breeding system describes the physiological aspects of compatibility between individuals involved in mating (Neal & Anderson 2005). In fungi, this would be the different mating types involved in sex. Mating-types in heterothallic (self-incompatible) ascomycetes are determined by two alternating mating-type alleles at a single locus. In filamentous ascomycetes, the term idiomorph (Metzenberg & Glass 1990) is often preferred over allele. This is because the sequences are very dissimilar even though they have been shown to be related by common descent (Martin et al. 2010). The locus is called $\textit{MAT1}$ and the idiomorphs $\textit{MAT1-1}$ and $\textit{MAT1-2}$ (Turgeon & Yoder 2000). This is called bipolar heterothallism, since the progeny of one ascospore are of two mating types (Debuchy et al. 2010). In contrast, in many heterothallic basidiomycetes the mating types are determined by two often multiallelic loci. They are thus said to be tetrapolar because the progeny of a single basidiocarp display four different mating types. In all known bipolar ascomycetes species only two different mating type alleles are present, with one exception in which there seems to be at least three alleles (Debuchy et al 2010). This can be compared to the basidiomycetes in which thousands of mating types are present in some species (Casselton 2002). Thus, to be able to mate, individuals carrying opposing mating types must meet (Ni et al. 2011). In homothallic ascomycetes both mating types are present in the same individual and it is therefore self-fertile. In homothallic species the term idiomorph is inappropriate (Debuchy et al. 2010) and instead the “alleles” are referred to simply as mating types. The mating type genes in homothallic species can be either linked or fused in the same locus; or unlinked, residing in two different loci (Ni et al. 2011). Quite recently it has also been discovered that some species can have sex even when only one mating type is present (Wilson et al. 2015). The mating type genes have names derived from the idiomorph in which they reside. The $\textit{MAT1-1}$ is thus the first gene described from the idiomorph $\textit{MAT1-1}$ etc. This gene together with $\textit{MAT1-2}$ are important for the identity of the mating types and are also master regulator genes in the sexuality of ascomycetes (Wilken et al. 2018). Often there are additional genes and usually the idiomorph is flanked by the conserved APN2 and SLA2 genes on each side (Fig. 3).
**Figure 2. Breeding systems and modes of sexual reproduction in fungi.**

**Heterothallism** can be either bipolar or tetrapolar. **Bipolar** implies that one MAT locus with alternating alleles/idiomorphs/mating types is present. Two individuals with opposite mating types must meet in order to mate. In **tetrapolar** fungi there are two MAT loci that often are multiallelic. Two individuals must have different mating types at both loci to be able to mate. In **homothallic** fungi some species are able to switch mating type. Species that have two nuclei with different mating types in one spore are said to be **pseudohomothallic**. Other homothallic species carry both mating types either fused or closely linked in the same locus. There are also examples of species in which the mating types genes are unlinked, residing in two different loci. Finally some species are able to mate even though there is only one mating type present, so called **unisexuality** (adapted from Ni et al. 2011).
Figure 3. In the sister class to the Laboulbeniomycetes, the Sordariomycetes, the MAT locus contain the mating type genes *MAT1-1-1* and *MAT1-2-1* which most often are physically linked to the flanking genes APN2 and SLA2. *MAT1-1-1* contains an α-box domain, whereas *MAT1-2-1* contains a related HMG-box domain. Some ascomycetes have an extra HMG-box (*MAT1-1-3*) that is also believed to be involved in sexual reproduction. The arrows indicate the direction of transcription (adapted from Palmer et al. 2014).
Thesis aims

Overall aims: why do we study Laboulbeniales?

First of all, parasites in general represent a huge diversity. It has been speculated that there may be more parasites than free living species (Agabian & Metzenberg 1992, Windsor 1998). Diversity aside, parasites also display a vast variety of lifestyles and life cycles which present great possibilities of comparative research both on specifically parasite related questions and on evolution in general (Criscione et al 2005). Parasitism has also been claimed to be a driving force in evolution and ecology (Haldane 1949, Kochin et al. 2010). Laboulbeniales could therefore be used as models to study evolutionary patterns. Questions regarding, e.g., host switching, intra-host speciation and cospeciation could all be addressed with Laboulbeniales and their hosts as models.

Specific aims

In this thesis I chose to look at the genus Coreomyces from different perspectives. In paper I, we use Coreomyces as a convenient and abundant representative of the Laboulbeniales in order to develop an efficient and reliable molecular methodology that served as a foundation for subsequent research. In the following paper (II) our goal was to clarify host and position specificity in the genus Coreomyces; the aims were firstly to test to what extent thalli growing in different positions on their corixid hosts correspond to species as independent evolutionary units as described by de Queiroz (2007), and secondly to assess the extent to which species display position specificity, host specificity, or sex of host specificity.

The aim for paper III was to describe and name the taxa distinguished in paper II (Sundberg et al. 2018b). We also show how position induced intraspecies morphological plasticity in the Laboulbeniales may confuse species delimitation.

Finally, in paper IV we wanted to determine whether the monoecious species in the genus Coreomyces are homothallic or heterothallic.
Summary of paper I

Until recently, there has been limited success using molecular approaches in research of Laboulbeniales. Few DNA sequences exist and every DNA sequence produced has generally consumed many individual thalli (e.g. Weir & Blackwell 2001a, b, Goldmann & Weir 2012, Haelewaters et al. 2015) and material is often scarce. Moreover, this prohibits genetic studies at population and individual level.

There are several factors impeding successful molecular work with these microscopic fungi. In the first step of preparing a sample there is a risk of losing the thallus once detached from the host integument, often due to static electricity. After the thallus is removed comes the problem of breaking up or lysing the cell walls. When the cell content is released, the minimal amount of DNA may get lost in the extraction process, especially when using standard DNA extraction kits. And finally, at the DNA amplification stage, it is believed that PCR-inhibiting substances such as melanin co-extracted in the DNA extraction step may interfere with the polymerase reaction (Goldmann 2015; Haelewaters et. al 2015).

In order to develop and improve the methods used we sampled species from the genera Coreomyces, Ecteinomyces, Herpomyces, and Laboulbenia. The material was preserved either in 99.7% EtOH or pure propylene glycol. To avoid the effects of static electricity after detachment, the thallus/thalli were ‘glued’ to the tip of a micropin with a droplet of sucrose and thereafter transferred to a decontaminated 3 mm diameter glass coverslip, another coverslip was put on top and the “coverslip sandwich” was transferred to a purpose built arbor press where the sample was crushed (Fig. 4). Most of the time we were able to use a single thallus per reaction, thus demonstrating that it is possible to perform analyses on an individual level.
Figure 4. The arbor press with custom-made components in shades of grey

Illustration of the arbor press (a) with the custom-made components. Two stainless steel discs (b) are used to crush a microscopic sample that is placed between two glass coverslips (c). The steel disc/cover slip, with the fungal thallus in between, is fitted into a depression in the titanium holder (d). The titanium holder is subsequently placed in a steel holder (e) centred under a titanium rod (f) inserted in the arbor press tool holder. The components denoted g and h are used to elevate the coverslips as described in Fig. 5. Note, the fungal thallus depicted is enlarged about seven times compared to the components.

After crushing, the coverslip sandwich was transferred to a 1.5 mL microcentrifuge tube (Fig. 5). The coverslips were covered with a mix of PCR kit buffer and PCR grade water in sufficient volume for the number of reactions wanted (min 2, max 4). The tube was then vortexed, the contents spun down and apportioned to PCR tubes, and the amplification reactions were set up.
Figure 5. Flowchart showing the different steps in the crushing process

Letters refer to the components outlined in Fig. 4.

1. Pressure is exerted on the steel discs and the glass coverslips (b + c) by the titanium rod (f) when lowered with the arbor press lever. After the fungal tissue has been crushed, the coverslips are pushed up from the depression in two steps using discs with centred pins (g, h) that differ slightly in length.

2. The shorter pin is inserted into the hole on the underside of the titanium holder (d).

3. The steel disc compound is pushed upward enough for the top steel disc (b) to be removed.

4. The longer pin is inserted.

5. The coverslips (e) with the crushed fungal tissue slide off the lower steel disc and are transferred to an Eppendorf tube for subsequent PCR.

We amplified four different loci: the ITS including 5.8S (roughly 800–1,100 bp depending on species), partial nrLSU (roughly 1,300 bp), partial nrSSU (roughly 1,100 bp) and partial mtSSU (between 680–740 bp depending on species). The total PCR success rate, excluding those that showed double bands on gel, was 89.4% (161 out of 180 reactions). A total of 92.8% of the
153 PCR reactions on *Coreomyces* extracts, 100% of the four PCR reactions on *Herpomyces* extracts, 75% of the four PCR reactions on *Ecteinomyces* extracts, and 63.2% of the 19 PCR reactions on *Laboulbenia* extracts were successful. The total number of generated sequences was 156 (56 ITS, 59 nrLSU, 21 mtSSU, 20 nrSSU).

We believe that our methodology may be widely applicable to any other minute and unculturable fungi with tough cell walls. The possibility to use individual thalli saves precious samples and also allows for, e.g., allele frequency studies or genetic studies at the level of an individual. Previous published methods were all prone to loss of material in the extraction procedure, this is a problem that our method eliminated.
Summary of paper II

Position specificity (Peyritsch 1875, Thaxter 1896, Benjamin & Shanor 1952, Whisler 1968) implies that two or more species share the same host species and that each parasite is confined to its own restricted position. This phenomenon is known from various parasites in the animal kingdom (Littlewood 1997; Martin 2004) but is unique to the Laboulbeniales among the fungi. A special case is so called sex-of-host specificity where a parasite is confined not only to a certain position but also to host sex. This type of specificity has, however, in the cases investigated so far, been shown to involve morphotype pairs of the same species taking different shapes in corresponding positions in male and female hosts (Goldman & Weir 2012; Goldman et al. 2013).

In this paper we focused on the genus *Coreomyces*. All of its species are parasites on water boatmen (Heteroptera, Corixidae) and display position specificity. The only more thorough study of the genus focused on *Coreomyces* specimens from Poland and surrounding countries (Majewski 1973, 1994); no molecular work has previously been carried out.

We sampled corixids from southern Sweden and Denmark, and were able to obtain DNA sequence data from thalli growing in different positions from numerous corixid species of both sexes.

Captured animals were immediately killed and preserved in 99.7% EtOH. Ten species of corixids in four genera were encountered. Thalli were found in three positions previously named: CV, LW, and LV. We found thalli in two positions previously not reported that we denoted RD and RV (Fig. 6). Subsequent sample processing and downstream molecular work followed the protocol in paper I. We used between one and six thalli from 76 corixid individuals. Sequences were produced for the following molecular markers: nrSSU (n=24), ITS (n=62), nrLSU (n=63) and mrrSSU (n=27). Newly produced sequences were edited and aligned together with previously published sequences (paper I).
Trees were created using maximum likelihood and Bayesian inference. For the maximum likelihood analyses, the best-fitting GTR family model was selected using the Bayesian Information Criterion (BIC). For the ITS data, we additionally selected the best-fitting time-irreversible RY Lie Markov model to be able to infer the placement of the root. (Sumner et al. 2012, Fernández-Sánchez et al. 2015, Woodhams et al. 2015) The laboulbeniomycete sequences publicly available proved too divergent to allow rooting, instead we chose to root using the information inside our data. For the Bayesian inference, we used the same best-fitting models as in the ML analyses. The trees generated (example in Fig. 2) shows four distinct clusters (named blue, orange, green and red). To delineate species we used single-rate Poisson tree process (PTP) and multi-rate Poisson tree process (mPTP) modelling to locate the transition points between inter- and intraspecific processes in the accumulation of substitutions on the individual gene trees (Zhang et al. 2013; Kapli et al. 2017). We also carried out a multinominal logistic regression analysis in R (Venables & Ripley 2002) with clade membership as the response variable and position on host, host sex and host genus.

The statistical analyses suggest what is obvious from looking at the phylogenetic tree in Fig. 7: There is a strong correlation between clade membership and the position of the fungus on the corixid host: Thalli in position LW came out as two sister groups (blue and orange), meaning that there seem to be two species occupying the same position (Fig. 6). All samples in position LV group in one clade (green). Thalli in position RV group into the red clade. The thalli in position RD appear inside two different clades in the tree (the green
and red). Finally, thalli from position CV belong to the red, orange and blue clades.

**Figure 7.** Inferred phylogeny based on ITS data under a RY6.7b time-irreversible model. Branch support is indicated (upper: bootstrap proportion in ML analysis, lower: posterior probabilities in the Bayesian inference). Crown groups have been transformed into triangular cartoons, the width of which represents the branch length from the most recent common ancestor to the tip of the highest branch, and the height of which is proportional to the number of included individuals. Clades have been annotated with the number of individuals in the five positions on the hosts (see Fig. 6 for an explanation of abbreviations).

The four major clades (green, red, orange, blue) were interpreted as species based on: 1) Poisson tree process modelling on the ITS and nrLSU trees indicates that the clades represent independent species, (2) they are topologically completely congruent across markers (although with different degrees of resolution), and (3) they correlate with ecological parameters, primarily the position on the host.
The Coreomyces species found in this study did not show any strict specificity to host species, which is in agreement with available information on host preferences (e.g., Thaxter 1931, Majewski 1994). Our results also indicate that none of the four species is restricted to only a single position on its host, although one of the positions tend to be much preferred over the other(s). The latter explains why the statistical analysis suggest a strong interaction between position and clade membership. This non-strict position specificity is in contrast to the conclusions from earlier studies (Majewski 1973, 1994). The statistical analysis also indicate that the distributions of positions are not identical between host sexes. The explanation to this is probably that thalli growing in positions CV and RV were only encountered in male corixids in our material. The other positions show a more equal distribution between the host sexes. The multinomial logistic regression did not reject the null hypothesis of no effect of host sex on clade membership, nor did the stepwise log-linear model selection indicate that interaction between host sex and clade membership is needed to explain the observed data. However, the multinomial logistic regression does suggest that host sex confers increased odds for membership in two of the clades. Sex-of-host specificity may be a non-existent extreme in a continuum, where instead weak preference for one host sex may turn out to be frequent.

The dispersal and positioning of the fungi are likely to be caused by host behaviour. Earlier studies (Goldmann & Weir 2012; Goldmann et al. 2013) have shown that parasites growing in the same position in both host sexes can be the result of a combination of hetero- and homosexual mounting by males and that all other positions stem from interactions between males and females during mating. Detailed descriptions of corixid mating (Peters 1962) and reports on homosexual mounting (Popham 1961, Aiken 1982) give us a hint of the mechanisms behind dispersal and positioning of members of the Coreomyces. The male swings his abdomen under the female on the left side. This behaviour may account for the positions to the left on the host. A peculiarity in male corixid morphology may also account for the unusual CV and RD positions. The male abdomen is not bilaterally symmetric and, moreover, both dextral and sinistral forms occur in many species, the former being much more common (Schilthuizen 2013). In sinistral forms, the copulation is consequently mirrored (Peters 1962). There are also other behaviours both during copulation (Jansson 1979) and agonistic, e.g. when males try to outcompete each other in courtship (Jansson 1973, 1979; Candolin 2004) that may play a role in the transmission of the fungi. Non-social behaviour (e.g. cleaning) resulting in fungi growing in the same positions in both host sexes as suggested by Huldén (1983) is an unlikely explanation to the patterns we see, as we would then expect to find thalli growing on the host limbs. The ‘male-only’ positions, however, may be accounted for by some male behaviour that actively transmits thalli from non sex-specific positions as suggested by Benjamin (1971). Passive infection through spore-contaminated environment, as
suggested for terrestrial species (Arwidsson 1946; Lindroth 1948), was also ruled out by the non-random positions of the thalli.

Our findings lead to further questions such as: How do species boundaries arise when host preferences are not discontinuous? How does position specificity arise and how is it related to substrate requirements? How does mating take place in the fungus, if at all? Genomic data as well as controlled observations of host behaviour under laboratory conditions may help solve these and other questions.
The genus *Coreomyces* belongs to the order Laboulbeniales (Goldmann & Weir 2018, Haelewaters 2018), which are mostly microscopic ascomycetes that are obligate parasites of arthropods. *Coreomyces* is a small genus with 21 described species, 20 of which were described in the beginning of the 20th century (Thaxter 1902, 1905; Spegazzini 1917, 1918; Thaxter 1918; Poisson 1929; Thaxter 1931) and one more recently (Lee & Na 2009). Four of these species have been recorded from the eastern and southern parts of Europe (Santamaria et al. 1991, Majewski 1994). In paper II, we found four phylogenetically distinct species in the genus *Coreomyces* that we provisionally named blue, orange, green, and red. To be able to identify these species, we used new material from localities in southern Sweden. Additional specimens from Turkey were added to see whether these were separated phylogenetically, although they displayed the same morphology as well as position on the host. Position on the host (in total 48 hosts) was documented before thalli were detached. Each thallus (n=88) was then photographed in order to be able to document measurements after the thallus had been consumed in the molecular work. DNA extraction and PCR followed the protocol by Sundberg et al. (2018a). Two markers (nrLSU and ITS) were used for confirmation of identity against sequences produced in Sundberg et al. 2018b. A set of measurements of each thallus that generated a sequence was recorded. The four taxa were compared with the type material of ten relevant species described by Thaxter (1902, 1905, 1918, 1931) and with the descriptions and illustrations of three relevant species described by Spegazzini (1917, 1918).

Two of the four species were described as new taxa, *C. confusus* sp. nov (Fig. 8) and *C. dextrorsus* sp. nov. (Fig. 9). These species are treated as the ‘orange’ and ‘red’ species, respectively, by Sundberg et al. 2018b). The former grows most frequently on the distal inferior margin of the left hemelytron (forewing) in many species of corixids, and the latter grows most frequently on the right lobe of the 8th sternite, near the inner margin, and on the adjacent middle lobe of the 7th sternite in several corixid species. The other two species encountered correspond to *C. macropus* and *C. corixae* (the ‘blue’ and ‘green’ species, respectively, of Sundberg et al. 2018b). These species are also reported as new to Denmark, Sweden and Turkey.
Figure 8. Coreomyces confusus

Thallus from the inferior side of the left hemelytron outer margin, with an extra (appendiculate?) cell with poorly developed lower septum inserted between cell I and II. Scale bar = 100 μm.

Figure 9. Coreomyces dextrorsus

a. Thallus of the abdominal form, with sterile branchlets attached to the appendiculate cells. Hyaline ascospores with gelatinous sheaths clinging to the peritheciun. b. Thallus of the dorsal form, with branchlets broken off. Scale bars = 100 μm.
Coreomyces macropus and C. confusus grow together on the hemelytron margin and occur rarely also in the middle of the 5th sternite on male hosts, where they adopt a different morphology more or less identical to C. arcuatus sensu Majewski (1994). The other two species, C. corixae and C. dextrorsus, are also encountered in positions other than the typical ventral. They then grow dorsally at the right margin of the male host abdomen: C. corixae on the 6th and 7th tergites, C. dextrorsus somewhat more posterior on the 8th tergite. These positions are not previously reported in literature. Although there are distinct morphological differences between thalli of C. corixae and C. dextrorsus growing ventrally, there seem to be no differences between dorsal forms of the two species, though only a single sample of C. dextrorsus was available.

Consequently, we have shown that the morphology of a species may vary with its position on the host. This agrees with the findings of other authors (Goldman & Weir 2012; Goldman et al. 2013; Haelewaters 2018). However, our research also indicates that different species may adopt the same or very similar morphology when sharing the same position, as C. confusum and C. macropus; or when growing in close proximity, as C. corixae and C. dextrorsus in their dorsal forms.

Under such premises, describing new species is a delicate task. How can one detect species-specific differences when neither morphology nor position is definite? And although the genetic composition differs, it is not always possible to perform these kinds of analyses, especially not on type material that is microscopic and mounted on a microscope slide. Nevertheless, we compared the newly described species with other species having similar morphology and/or growing in the same positions. These comparisons are often problematic, since old descriptions are often imprecise when it comes to position on the host, host species, and host sex. Moreover, they are often based on a scarce material, sometimes coming from a single host individual. As has been experienced in other fungal groups, we may therefore expect future studies in the Laboulbeniales to result in the discovery of cryptic diversity, whereby species diversity estimates may increase, as well as intraspecific variation in the phenotype, reducing those estimates.
Summary of paper IV

From the presence of antheridia and perithecia and from the lack of known anamorphs it has been assumed that the Laboulbeniales reproduce only sexually (Tavares 1985). This view has been further strengthened by observations of trichogynes in close contact with or growing towards antheridia (Benjamin & Shanor 1951, Weir & Beakes 1996). However, there are no observations of spermatia entering the trichogyne or ultimately plasmogamy. Therefore sexuality in Laboulbeniales must still be regarded as a hypothetical, although most likely, condition.

In the monoecious species, male and female sexual structures are found in the same thallus, whereas they are separated in the dioecious species. In the latter, spore pairs of opposing sex, but from the same ascus, are deposited together and develop simultaneously (Richards & Smith 1955, Benjamin 1971). Thus, the most obvious interpretation is that monoecious species are homothallic and predominantly selfing (Weir & Beakes 1996). The dioecious species, on the other hand, are likely to be heterothallic and prone to intratetrad mating, i.e. sex between two haploid individuals resulting from the same meiotic event (Zakharov 2005).

To be able to infer homo- or heterothallism in some members of the genus Coreomyces, we collected hosts that were subsequently scanned for infection. Five thalli from the left wing margin of five individual hosts were selected for subsequent molecular work. In order to avoid sampling more than one genome only immature thalli, without mature ascospores, were chosen. Thalli were cut at the appendiculate cells and only the lower part was used. This was to further ensure that there were no other genomes present in the sample.

DNA release followed the protocol of Sundberg et al. 2018a. The total genomic DNA from each individual was amplified with a single cell multiple displacement amplification (MDA) kit. The amplified genomes were then sequenced on the Illumina HiSeq X platform. Potential contamination of genomic DNA other than from the target genome was assessed by evaluating the k-mer composition. Rare k-mers with deviating GC-content were filtered out and the genomes were then assembled.

Mating and flanking gene sequences in each genome were searched for using the BLAST+ suite. We used representatives of Sordariomycetes as queries, because they are the closest relatives to the Laboulbeniomycetes. Scaffolds from two genomes containing hits for the targeted genes were aligned in order to
get an indication of the architecture of the MAT locus, even though the assembly software was not able to connect the genes in the individual genomes.

The KAT analysis revealed that all five genomes contain an abundance of rare k-mers but only one large peak of common k-mers (exemplified by genome #1 in Fig. 10). Searches for the MAT loci with tblastn provided hits for the MAT-1-1-1 gene in all five genomes and in all but genome #1 for the MAT1-2-1 gene. We also recovered the SLA2 gene from all five genomes. APN2, on the other hand, was only encountered in genome #1 and #4. The alignment of the scaffolds containing hits for the mating and flanking genes from genome #3 and #4 showed that the scaffold containing MAT1-1-1 and the one containing MAT1-2-2 are in close proximity to each other (Fig. 11), and also that SLA2 is connected to MAT1-1-1. APN2 was not possible to align.

**Figure 10.**

KAT 27-mer frequency histogram from genome #1, which shows one distinct peak. This histogram describes the sample of reads after digital normalization but before we filtered out rare 27-mers. Histograms from the four other genomes look virtually identical.

Our results indicate that the two species of *Coreomyces* included in the study, *C. macropus* and *C. confusus*, are homothallic. We base this conclusion on the findings of both MAT1-1 and MAT1-2 in long scaffolds with high sequencing depth in four out of five genomes. KAT analyses indicate that these results are not artifacts caused by the presence of more than one genome in the target organism, as we would then have expected one or more evenly spaced peaks to the left of the main peak in the k-mer frequency histogram (Fig. 10).
An alignment (using the de novo assembly tool of Geneious 7.1.9) including sequences from both genome 3 and 4. The blast hits for $MAT1-1-1$ should be in the same position in the overlapping parts marked with a red circle and the $MAT1-2-1$ should be in the same position in both genomes somewhere to the left of the red line. Consequently, if this is true, these mating genes reside on the same chromosome but there is a minimum distance of 50 kbp between them.

We have not been able to definitely confirm how the mating genes are positioned, i.e. if there is a linkage between them, or if they reside on different chromosomes. However, the alignment including sequences from genome #3 and #4 (Fig. 11) shows that there seems to be a relatively small gap (1396 bp) between the scaffold containing $MAT1-1-1$ and the one containing $MAT1-2-1$ in genome #4. This gap is overbridged by a scaffold from genome #3. If this gives a true picture of the mating type architecture, it is apparent that the MAT genes reside on the same chromosome, but that they are separated by more than 50 kbp. We were not able to connect APN2 to $MAT1-2-1$, which could indicate that the former resides at another position in the genome.

We conclude that one important reason for maintaining homothallism in the genus *Coreomyces*, and other selfing species of Laboulbeniales, is probably the advantage it confers in colonising new hosts, without the need for a partner. Another reason to maintain the costly sexual machinery under selfing, even though it does not provide any recombinational advantage (apart from DNA repair) over asexuality, could be the possibility of rare outcrossing (Lee et al. 2010). There are also a number of other possible advantages accounted for by Silliker et al. 1996, Coenen et al. 1997, Bruggeman et al. 2003, Galagan & Selker 2004 and Aanen & Hoekstra 2007. The main reason, though, can be the requirement of ascospores to be able to infect a host (Heitman 2010). Selfing then assures that ascospores are produced in absence of a partner. This in turn may provide a possible explanation to the retention of sexuality in the transition from a life cycle that alternates between an anamorphic and a teleomorphic state, as in the imagined ancestor of Laboulbeniales (Blackwell et al. 1986, 1994), to one that relies entirely on sex. This ancestor is assumed to have had a life cycle similar to that of the Pyxidiophorales, which is the sister group of the Laboulbeniales. Fungi in this order alternate between a mycoparasitic or saprobic sexual stage and an anamorphic stage tied to arthropods. When the hypothetical transition from a heteroxenous to a monoxenous life
style took place in a hypothetical ancestor to Laboulbeniales, it was crucial to be able to produce ascospores to maintain the ability to infect new host individuals. Since it is the anamorph that is tied to the arthropod in *Pyxidiophora*, physiological properties of the anamorph (being able to attach to the host) needed to be retained, but there was also a need for a shift from asexuality to sexuality in this phase of the life cycle. Selfing (or spore pair formation as in dioecious species) would, in the light of this, be favoured, since it allows for host colonisation from a single dispersal unit and for continuous infection when chances of outcrossing are restricted.

Selfing may also be an important clue to how multiple closely related species of *Coreomyces* can inhabit the same host species. An individual of *Coreomyces* accidentally colonising a new position on the host, a new host species, or a new geographic range will potentially be subject to strong selective pressure (Huyse et al. 2005), yet has all the resources needed to reproduce through self-fertilisation.
Conclusions and future prospects

In this thesis we have made substantial progress in the molecular techniques much needed for advancing the study of the enigmatic order Laboulbeniales. The techniques developed in paper I make the first steps towards studying the genetics of this order at both individual as well as population level. This has allowed us to obtain evidence aiding the understanding of the phenomenon of position specificity as described in paper II. Our work in paper III with species delimitation and taxonomy in the genus *Coreomyces* has also pin-pointed some of the difficulties we face in trying to understand the Laboulbeniales, for example that different species adopt a similar morphology when growing in the same position on the host. In paper IV we used molecular analysis to investigate mating systems in the genus *Coreomyces*, showing that monoecious species most likely are homothallic and that selfing probably confers an evolutionary advantage, which may be an important clue as to why several closely related species share the same host.

Future research in the field should address methodological problems such as amplification and sequencing of single copy genes. Such an ability is necessary in order to produce well supported phylogenies. Given the limited amounts of DNA available in most cases, PCR amplification may not be possible. Whole genome amplification may provide a solution, but has its own technical difficulties in addition to being costly. Something that also would be desirable is the development of techniques to be able to produce sequences from museum material, where DNA is likely to be degraded. Since samples of fresh material from many species and even genera in the Laboulbeniales may prove to be difficult to obtain, material from museums may be the only practical way to obtain genetic information so as to include these taxa in future phylogenies.

We should also continue to explore the mating types of Laboulbeniales in order to better understand their mode of reproduction. The next step in continuing the study of *Coreomyces* described in paper IV, would be to definitively show whether or not the mating-type genes are linked. When this is done we can annotate the genes to provide the full picture of the mating type architecture.
I december 1772 höll Uppsala universitets rektor, Carl von Linné, ett tal i Uppsala domkyrka om "Deliciae Naturae", naturens läckerheter. Han uttryckte där tydligt sin uppfattning om svampars värde och betydelse: "Svamparne äro ett ströfvande pack, som röfva alt hvad de finna lemnat, sedan Flora gådt i sit Vintertält emot Hösten". Efter det kunde svamparnas anseende bara bli bättre...

Men vad är då en svamp? De ansågs länge vara ett slags primitiva, klorofylllösa växter, och inte förrän i slutet av 60-talet upprättade ett eget rike för dem. Det dröjde sedan ytterligare ett antal år innan detta vann fotfäste inom vetenskapen och så småningom blev etablerat i de breda folklagren. Sedan dess har vetenskapen visat att svamparna står närmare djuren än växterna och det står allt klarare att de utgör en av de allra artrikaste och ekologiskt mest betydelsefulla organismgrupperna. Antalet beskrivna arter uppgår idag till omkring 100 000, men den totala globala artrikedomens antas vara betydligt högre. Olika uppskattningar under de senaste decennierna har varierat mellan 1,5 till 5 milioner arter, eller till och med ännu mer.


I min forskning har jag inriktat mig på en dåligt känd grupp av sporsäcksvampar (inom sporsäcksväxterna ryms bl.a. murklor och tryfflar), de dåligt befordrade och tungvrickande laboulbeniaer (ordningen Laboulbeniales), en grupp med många unika egenskaper. De utgör en artrik grupp som med få undantag är mikroskopiska (vanligen mellan 0,1–0,5 mm) och som lever som...
obligata (de klarar sig inte utan sin värd) men relativt harmlösa parasiter på leddjur, främst skalbaggar. Det är också en av de minst undersökta svampgrupperna, något som delvis kan förklaras av att mykologer sällan bryr sig om insekter och att entomologer sällan tagit notis om de svampar som är knutna till insekter och andra leddjur.

Laboulbeniales uppvisar olika specificitetsnivåer beroende på art. Detta kan grovt översättas till olika grader av inskränkthet vad gäller val av värdjur, rumslig utbredning o.s.v. Många arter håller sig till ett specifikt värddjursläkte och i extremfallet endast till en art inom släktet. Hos ett antal laboulbenialesarter är specialiseringen så långt driven att de bara växer på en viss del av värddjuret, t.ex. höger täckvinge. I en del fall samsas flera sådana "positionsspecifika" arter om samma värd och sitter då i princip bara på sin ”egen” position.


I min forskning har jag strävat efter att kasta nytt ljus över positionsspecificiteten och artgränserna inom släktet Coreomyces. Alla medlemmar i detta släkte är obligata (måste ha sin värd för att klara sig) parasiter på buksimmare (små vattenlevande insekter) och har ansetts växa på sin bestämda position, som dock i vissa fall delas med andra arter ur släktet. Det har dock ifrågasatts om det i alla fall verkligen rör sig om skilda arter, eftersom det enda som egentligen skiljer vissa arter åt är deras storlek och form, egenskaper man ansett påverkas av positionen.

För att kunna ta reda på hur det verkligen förhåller sig har jag använt mig av metoder som går ut på att analysera svamparnas DNA. Eftersom dessa svampar är mikroskopiska och dessutom inte går att odla är detta en grannlaga uppgift. Jag har därför varit tvungen att utveckla en tillförlitlig teknik (se artikl I) för att extrahera DNA från enskilda svamper. För att komma åt DNA...
har jag utvecklat ett sätt att helt mekaniskt frigöra cellinnehållet. Andra har försökt använda bl.a. flytande kväve och olika enzymer med måttlig framgång. DNAt har sedan amplifierats (föröks) med den så kallade PCR-tekniken för man skall få tillräckligt med DNA för att kunna sekvensera (läsa) det. Att kunna sekvensera DNA från en enda svamp är viktigt om man vill kunna studera enskilda individers genetik men också för att kunna förstå olika genetiska mönster inom en population. Eftersom många som arbetar med små icke-odlingsbara organismer i många avseenden står inför samma problem (få och små individer, små mängder DNA etc.) har den metod som jag tagit fram en potential att vara mer allmänt tillämplig.


Ytterligare en okänd position uppdagades, till höger på bukens ovansida. Här visade det sig att arterna från bukens undersida hade ett alternativt tillhåll. Denna position, liksom mittpositionen på bukens undersida, verkar vara förbehållen hanar av värddjuret. En tänkbar förklaring till detta kan tänkas vara den speciella parningsställningen hanarna intar i kombination med homosexuellt beteende och det faktum att vissa hanar har spegelvända genitalier (för en detaljerad redogörelse se artikel II).

Syftet med den fjärde artikeln var att avslöja parningssystemet hos vingkantsarterna *Coreomyces confusus* och *Coreomyces macropus*. Arterna inom släktet är alla sambyggare/monoika (d.v.s. de har både han- och honorgan inom samma individ). Vad jag ville ta reda på var om de i och med detta även är självfertila, vilket inte är helt uppenbart (jfr äpplen som måste korsas med andra äppelsorter för att sätta frukt trots att blommorna är tvekönade). Självfertilitet kallas i svampsammanhang för homothallism. För att kunna ta reda på hur det förhöll sig, var jag tvungen att amplifera upp hela genomet (lite förenklat, allt DNA i en cell). Detta eftersom jag inte på förhand visste var i
ärvsmassan jag skulle leta efter de gener som styr om en art är självfertil eller ej. När jag väl hade hela ärvsmassan sekvenserad använde jag kända sekvenser (av de gener som styr sexualiteten) från andra relativt närbesläktade svampar till att (starkt förenklat!) jämföra med mina svampars ärvsmassa. På detta sätt lyckades jag identifiera Coreomyce confusus och Coreomyces macropus som vardandes homothalliska. Med utgångspunkt i detta fördes sedan en diskussion kring vad självfertilitet får för konsekvenser för individen och arten och vad det finns för nack- respektive fördelar för en parasitisk svamp att kunna para sig med sig själv (se artikel IV för mer information).
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